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Reversal of Calcium Cycling Defects in Advanced Heart Failure

Toward Molecular Therapy

Masahiko Hoshijima, MD, PHD,*† Ralph Knöll, MD, PHD,* Mohammad Pashmforoush, MD, PHD,* Kenneth R. Chien, MD, PHD,*‡

La Jolla, California; and Boston, Massachusetts

Heart failure is a growing major cause of human morbidity and mortality worldwide. A wave of new insights from diverse laboratories has begun to uncover new therapeutic strategies that affect the molecular pathways within cardiomyocytes that drive heart failure progression. Using an integrative approach that employs insights from genetic-based studies in mouse and humans and in vivo somatic gene transfer studies, we have uncovered a new link between stress signals mediated by mechanical stretch and defects in sarcoplasmic reticulum (SR) calcium cycling. An intrinsic mechanical stress sensing system is embedded in the Z disc of cardiomyocytes, and defects in stretch responses can lead to heart failure progression and associated increases in wall stress. Reversal of the chronic increases in wall stress by promoting SR calcium cycling can prevent and partially reverse heart failure progression in multiple genetic and acquired model systems of heart failure in both small and large animals. We propose that reversal of advanced heart failure is possible by targeting the defects in SR calcium cycling, which may be a final common pathway for the progression of many forms of heart failure. (J Am Coll Cardiol 2006;48:A15–23) © 2006 by the American College of Cardiology Foundation

For heart failure patients and their physicians, these are the best of times and the worst of times. Over the past 3 decades, there have been major advances in the treatment of heart failure, ranging from an extensive list of medical therapy (beta-blockers, angiotensin-converting enzyme inhibitors, and so on), improvements in heart transplantation with new agents to suppress tissue rejection, and novel device technology (synchronized pacing/left ventricular assist devices, and so on). At the same time, despite these substantial clinical advances, heart failure has become the major cause of human cardiovascular morbidity and mortality worldwide, and has been predicted to reach epidemic proportions in the early period of the 21st century. The reasons for this clinical conundrum are undoubtedly multifactorial, reflecting the increasing lifespan, globalization of calorie-rich eating habits, the higher incidence of metabolic diseases and associated risk for ischemic heart disease, and the substantial improvement of the survival rate of acute coronary events. In fact, an exponential increase in the incidence of heart failure in China and India, followed by many other countries, is signaling an emerging global health problem of unprecedented proportions (1,2). At the core of this epidemic is our fundamental lack of understanding of the precise molecular pathways that drive human heart failure (i.e., mechanisms underlying the progressive dilation of the cardiac chamber and the associated decreases in cardiac contractility). However, several laboratories have successfully utilized an integrative approach to dissect pathways that lead to dilated cardiomyopathy (DCM), employing genetic-based studies in mice and humans, in vivo somatic gene transfer, bioinformatics, advanced imaging technologies, and computational biology, which is beginning to provide new insights into the disease process, and suggesting new therapeutic targets and strategies for intervening in the disease (3–7). Accordingly, a growing body of evidence suggests that a major component of this process now appears to be chronic increases in wall stress that trigger specific stress-related signaling pathways for critical cell responses, which include cell death pathways, survival cues, hypertrophic responses, and associated changes in the downstream pathways (8,9). In this review, we describe recent advances that have lead to the discovery of a pivotal role of defects in calcium cycling in the pathogenesis of human heart failure and the background that has led to a novel therapeutic strategy for reversal of these defects that will be slated for clinical studies in the coming year.

CHARACTERIZATION OF A GENETICALLY BASED MODEL OF DCM AND HEART FAILURE IN MUSCLE-SPECIFIC LIM PROTEIN (MLP)-DEFICIENT MICE

One of the first genetic links between cardiac cytoskeletal defects and DCM was made via studies of mutant mice that harbor a deficiency in MLP (10). Muscle-specific LIM protein, also known as cysteine-rich protein 3 or cysteine- and glycine-rich protein 3 (CSRP3), is a member of the

From the *Institute of Molecular Medicine and †Center for Research in Biological Systems, University of California San Diego, La Jolla, California; and the ‡Cardiovascular Research Center, Massachusetts General Hospital, Department of Cell Biology, Harvard Medical School, the Harvard Stem Cell Institute, Boston, Massachusetts. Supported by the Jean Leducq Foundation and an AHA National Scientist Development Award and NIH/NHLBI (HL081401) to Dr. Hoshijima. Jeffrey A. Towbin, MD, served as guest editor for this article.

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Abbreviations and Acronyms

BNP	= brain natriuretic peptide
CM	= cardiomyopathic
CSRP3	= cysteine- and glycine-rich protein 3
DCM	= dilated cardiomyopathy
HCM	= hypertrophic cardiomyopathy
MLP	= muscle-specific LIM protein
PKA	= protein kinase A
PLN	= phospholamban
post-MI	= post-myocardial infarction
rAAV	= recombinant adeno-associated virus
RyR	= ryanodine receptor
SERCA2	= sarcoplasmic reticulum calcium ATPase 2
SR	= sarcoplasmic reticulum
T-cap	= titin-cap
TTN	= titin

C-reactive protein gene family (11) and contains 2 highly conserved LIM domains that have been shown to serve as protein-protein interaction modules in a series of LIM proteins (12). Muscle-specific LIM protein is expressed in cardiac and skeletal muscle (10,13) and is predominantly localized in the cardiac cytoskeleton, mainly adjacent to the Z disc structure (10,14). Mutant mice with the genetic ablation of MLP display a cardiac phenotype that closely resembles human DCM, including progressive enlargement of all 4 cardiac chambers, ventricular wall thinning, decreases in cardiac contractility (10,15), induction of embryonic gene markers, defects in sarcoplasmic reticulum (SR) Ca^{2+} handling (15,16), with the elongation of action potential duration and the desensitization of beta-adrenergic signaling (10,17) (Fig. 1). In addition, genetic defects of CSRP3/MLP (18–20) and abnormal CSRP3/MLP expression (21) have been linked to cardiomyopathy and heart failure in human patients. Thus, the MLP-null mouse serves as an ideal tool for unraveling pathways that lead to heart failure initiation and progression, as well as the identification of new therapeutic strategies via genetic complementation.

AN MLP/TITIN-CAP (T-CAP)/Z DISC TITIN COMPLEX CONSTITUTES AN ESSENTIAL COMPONENT OF THE INTRINSIC MECHANICAL STRESS SENSOR MACHINERY IN CARDIOMYOCYTES

Utilizing the MLP-null mouse model of cardiomyopathy, we revealed a critical role for MLP as an essential component of the cardiac muscle stretch sensor (18). A combinatorial approach of yeast 2-hybrid screening of a cardiac cDNA library, glutathione S-transferase-pull down, and immuno-co-localization analyses identified a 19-kD cytoskeletal protein, T-cap (T-cap: telethonin), as a MLP-binding protein. This molecule localizes at the sarcomere Z disc and binds to the NH_2-terminal Z disc domain of the titin molecule (Fig. 2). The T-cap interacting domain of MLP was identified in a short NH_2-terminal domain of MLP that precedes the first LIM domain (18). Immuno-staining of T-cap in the MLP-null myocardium demonstrated that T-cap was dissociated from Z disc and diffused out in the cytoplasm in a fraction of cardiomyocytes, indicating the possibility that MLP stabilizes T-cap interaction with the NH2-terminal Z disc-associated domain of titin. In addition, electric-microscopic examination of MLP-null myocardium demonstrated that the MLP-null Z disc was widened, brushed, and wavy compared with wild-type control animals.

Titin serves a structural role in cardiac muscle in sarcomeric organization and assembly, as well as playing a crucial role in restoring cardiac sarcomeric length and in aligning the M-band at the center of the sarcomere during each

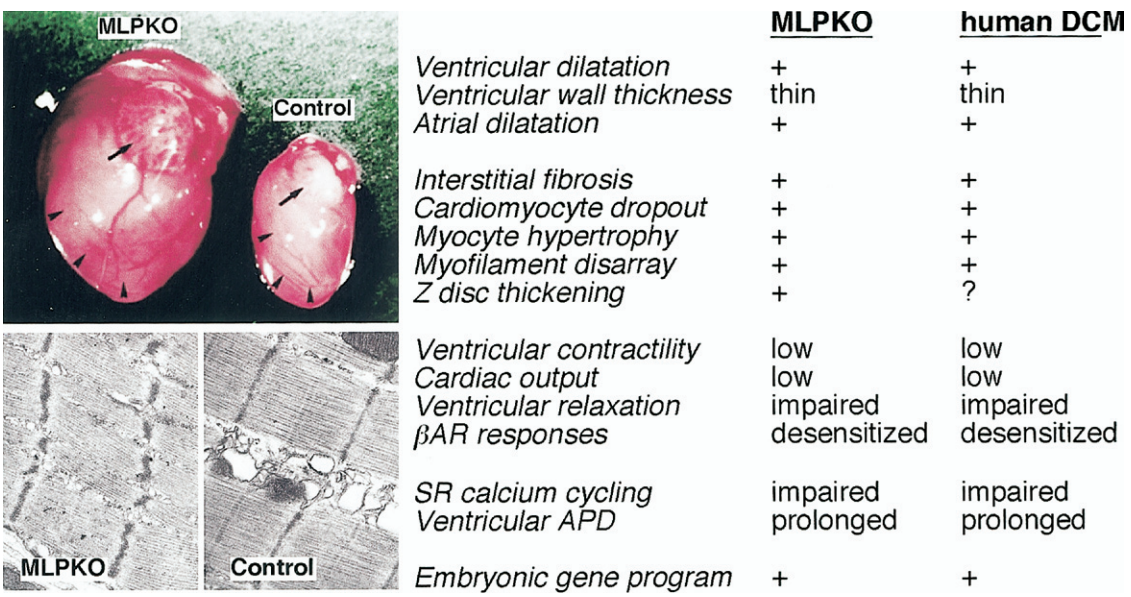

Figure 1. Muscle-specific LIM protein (MLP)-deficient (MLPKO) mice share a broad spectrum of pathological phenotypes with human dilated cardiomyopathy (DCM). Note defective Z disc found in the MLP-null myocardium. APD = action potential duration; SR = sarcoplasmic reticulum; βAR = beta-adrenergic receptor.

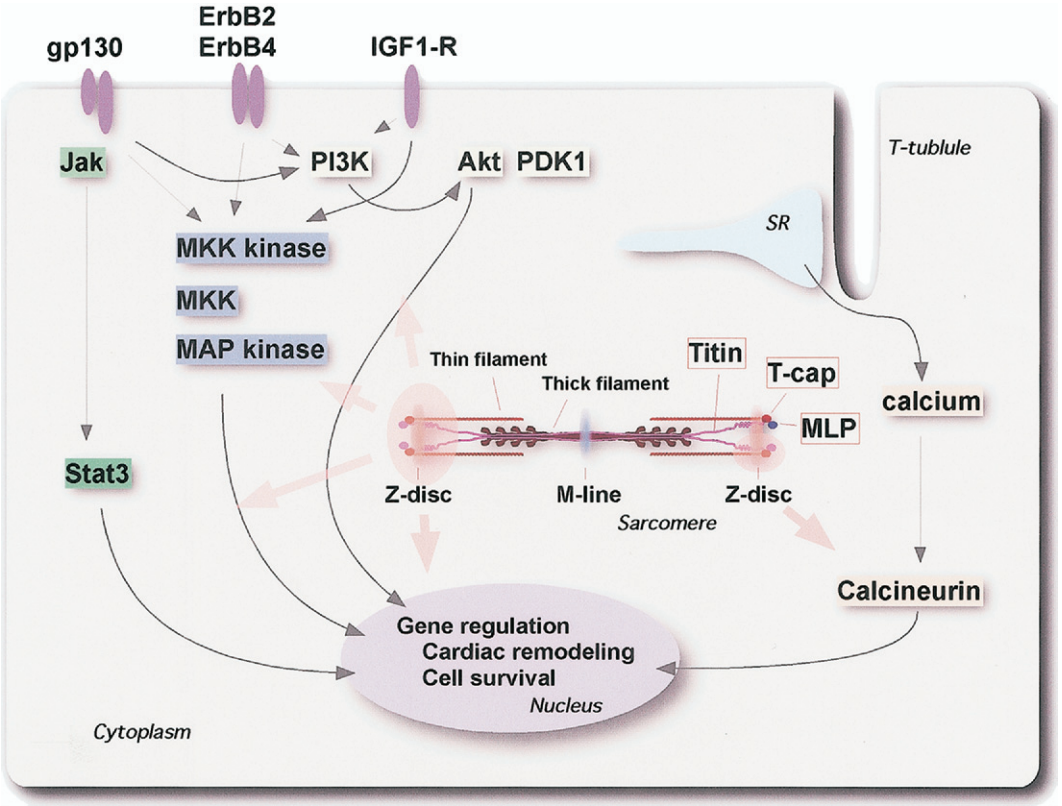

Figure 2. Z disc-related mechanical sensor cross-talks with receptor-dependent signaling. Transmembrane receptors including gp130 cytokine receptors, erbB2-erbB4, and insulin-like growth factor-1 receptor (IGF1-R) activate multiple signal kinase cascades that regulate cell protective gene programs. A calcium-activated calcineurin dephosphorylates multiple cellular substrates including transcriptional regulators that regulate cardiac remodeling. Cardiomyocytes, on the other hand, have (perhaps multiple) mechanical stress sensors inside of the cells, and the titin-T-cap-muscle-specific LIM protein (MLP) complex constitutes such a sensor systems associated with Z discs (24). Akt = protein kinase B; Jak = Janus kinase; MAP = mean arterial pressure; MKK = mitogen-activated protein kinase kinase; PDK1 = phosphoinositide-dependent protein kinase 1; SR = sarcoplasmic reticulum; Stat3 = signal transducer and activator of transcription-3.

cardiac contraction (22,23). Titin is anchored at the Z disc with its NH_2-terminal strongly associated with the actin thin filament that is cross-linked by α-actinin. Interestingly, in vitro physiological measurements revealed that MLP-null cardiac papillary muscles from 2-week-old MLP-null mice (before the development of general cardiac dysfunction), displayed a selective defect in the mechanical stretch properties of titin, with impairment in tension generation starting at a short range (within 10%) of passive stretch (18). Furthermore, cultured MLP-deficient neonatal day 1 to 3 cardiac muscle cells displayed a nearly complete loss of passive stretch-dependent induction of a genetic marker of cardiac mechanical stress, brain natriuretic peptide (BNP) (18). Importantly, the same MLP-null cardiomyocytes retained a normal BNP induction in response to G-protein coupling membrane receptor stimulation, providing direct support for a selective defect in mechanical stress sensing functions in MLP-null cardiac muscle. Taken together, our studies in MLP-null cardiomyocytes revealed that an MLP interaction with T-cap at the Z disc is critical to maintain the mechanical elastic properties of titin and that MLP/T-cap/Z disc-titin complex constitutes a critical molecular component of the cardiac muscle stretch sensor (24). In this manner, a loss or dysfunction of this intrinsic mechanical stress sensing machinery linked to the Z disc-titin structure may lead to the development of heart failure and DCM via the loss of the ability to activate stretch-mediated survival cues. Such survival cues could occur via the gp130-dependent, neurgulin-ErbB2/ErbB4-induced, or insulin-like growth factor-1-activated cardiomyocyte survival pathway (25–29) (Fig. 2), or calcineurin-dependent pro-hypertrophic nuclear gene regulatory pathway (30).

CSRP3/MLP MUTATIONS ASSOCIATED WITH FAMILIAL DILATED AND HYPERTROPHIC CARDIOMYOPATHIES (HCMS)

Since the discovery of the MLP mouse model of DCM, a growing list of genetic defects have been found in cytoskeletal proteins (7,31), as well as in extracellular matrix proteins that serve an anchoring function with the sarcolemmal cytoskeletal complex (32), linking them to the familial forms of cardiomyopathies. CSRP3 (human MLP) is encoded by an approximately 20-kb genomic sequence with 6 exons. We started with sequencing the CSRP3 coding exons of genomic DNAs obtained from selected DCM patients, followed by polymerase chain reaction–based selective single nucleotide change analyses for 536 well-phenotyped

Caucasian patients bearing familial DCM together with 320 control individuals with the same demographic distribution. Consequently, we identified a close association between 10 of 536 human DCM patients and a sense nucleotide alteration of CSRP3 (i.e., CSRP3[W4R]) in a highly conserved amino acid residue within the N-terminal T-cap binding domain (18). The linkage analysis in these families is limited by several factors, including the size of available pedigrees and age-dependent penetrance. Interestingly, the haplotype analysis supported a founder effect in a DCM patient population with CSRP3(W4R), although there were no linking records of affinity between patient families.

After the publication of our original report, at least 3 studies have analyzed CSRP3(W4R) in DCM and HCM patients. One study found CSRP3(W4R) in a family with DCM; however, CSRP3(W4R) did not segregate with DCM in this family (20). In another study, CSRP3(W4R) was found in 7 of 389 unrelated HCM patients with 1 in 400 reference alleles, and, interestingly, 3 HCM patients with CSRP3(W4R) also carried mutations of either beta-myosin heavy chain or myosin binding protein-C (33). Finally, a Canadian study reported 1 HCM patient with CSRP3(W4R) of 192, whereas CSRP3(W4R) was also found in 3 of 250 control individuals, who are predominantly of British Isles origin and did not receive formal cardiologic examination (34). Accordingly, it is plausible that CSRP3(W4R) is a genetic modifier or a relatively rare polymorphism that segregates with a subset of the cardiomyopathy population, rather than a disease-causing variant. Besides the W4R variant, other nucleotide changes (L44P, S54R/E55G, C58G, K69R) in the CSRP3 gene have recently been reported in patients with cardiomyopathies (19,20). The amino acid positions of these additional mutations are outside of the T-cap interacting domain of CSRP3.

Although genetic evidence is somewhat obscure, there was a strong biological finding to support the role of the CSRP3(W4R) amino acid substitution in the pathogenesis of DCM. The CSRP3(W4R) resulted in the complete loss of T-cap interaction shown by the yeast 2-hybrid analysis, and myocardial biopsies from a DCM patient harboring a CSRP3(W4R) allele showed partial T-cap dissociation from the Z disc. The biological significance of MLP/T-cap interaction in the maintenance of normal cardiac function was further emphasized by our independent identification of a single proband with the R87Q mutation of TCAP (human T-cap gene) in a set of Caucasian DCM patients (18). The R87 amino acid residue was mapped within the MLP-interacting domain of T-cap, and TCAP(R87Q) mutant protein displayed a ~80% reduction in binding affinity to MLP (35). Interestingly, other mutations in TCAP are linked to Limb-Girdle Muscular Dystrophy type 2G (36), suggesting that T-cap interaction with the Z disc-titin structure is requisite to maintain physiological functions of both cardiac and skeletal muscle. Herein, human titin (TTN) mutations have been linked to familial DCM (37,38), and a mutation was also found in an HCM patient (39). As a recent study proposes a model of N-terminal titin assembly that is mediated by the dimerization of T-cap in an antiparallel manner (40), we speculate that mutations of CSRP3, TCAP, and TTN share a common molecular pathway to induce and/or modulate the development of cardiomyopathy.

IDENTIFICATION OF SR CALCIUM CYCLING AS A MAJOR TARGET TO TREAT DCM AND HEART FAILURE: PHOSPHOLAMBAN (PLN) ABLATION RESCUES HEART FAILURE IN THE SUBSETS OF MOUSE MODELS OF DCM

Defects in SR Ca^{2+} cycling are a highly conserved signature feature of experimental and human heart failure. The most notable abnormalities are a decrease in the Ca^{2+} re-entry mechanism into the SR, which is predominantly governed by the activity of the SR calcium ATPase 2 (SERCA2) and dysregulated Ca^{2+} release from SR through ryanodine receptor (RyR) (Fig. 3A). In both cases, the SR calcium level is largely diminished in failing hearts, creating defects in both diastolic and systolic functions. In this manner, a decrease in the quantal release of calcium from SR through RyR during systole affects the contractility of individual cardiomyocytes, whereas the impaired calcium pump function results in a defect in ventricular relaxation. Cardiac muscle cells have an endogenous inhibitor of SERCA2, namely PLN, which is a highly conserved 52-amino-acid peptide and the expression of which is largely restricted in the heart and slow skeletal muscle. Phospholamban is phosphorylated by the cyclic adenosine monophosphate (AMP)-dependent protein kinase (PKA) that is regulated by G-protein coupling receptors including β-adrenergic receptors, and this phosphorylation in turn releases the PLN inhibitory effects on the SERCA2 activity (41,42). In many forms of human and experimental heart failure, the down-regulation of the SERCA2 expression level, impairments in the SERCA2/PLN ratio, and a decrease in the phosphorylated form of PLN have been documented (42–44). Whether these changes are part of abnormal cardiac remodeling in the development of heart failure or represent an adaptive process to limit cardiac function and reduce cardiac energy consumption to protect cardiomyocytes has been a point of discussion. To address this question, we utilized a genetic complementation strategy, which ultimately provided strong evidence that defects in SR Ca^{2+} cycling play a pivotal role in heart failure progression. We generated double knockout mice, which harbor the MLP-null cardiomyopathic mutation, but also lack PLN, and subsequently examined whether removing PLN inhibition alone would have a measurable effect on heart failure progression. Remarkably, the ablation of PLN completely prevented the spectrum of heart failure phenotypes found in a mouse model of DCM caused by MLP deficiency (16).

Subsequent studies designed to test PLN ablation in various forms of cardiomyopathy and heart failure reported mixed results (45–47). In some forms of transgenic cardiomyopathy mouse models, PLN-null rescued cardiomyopa-

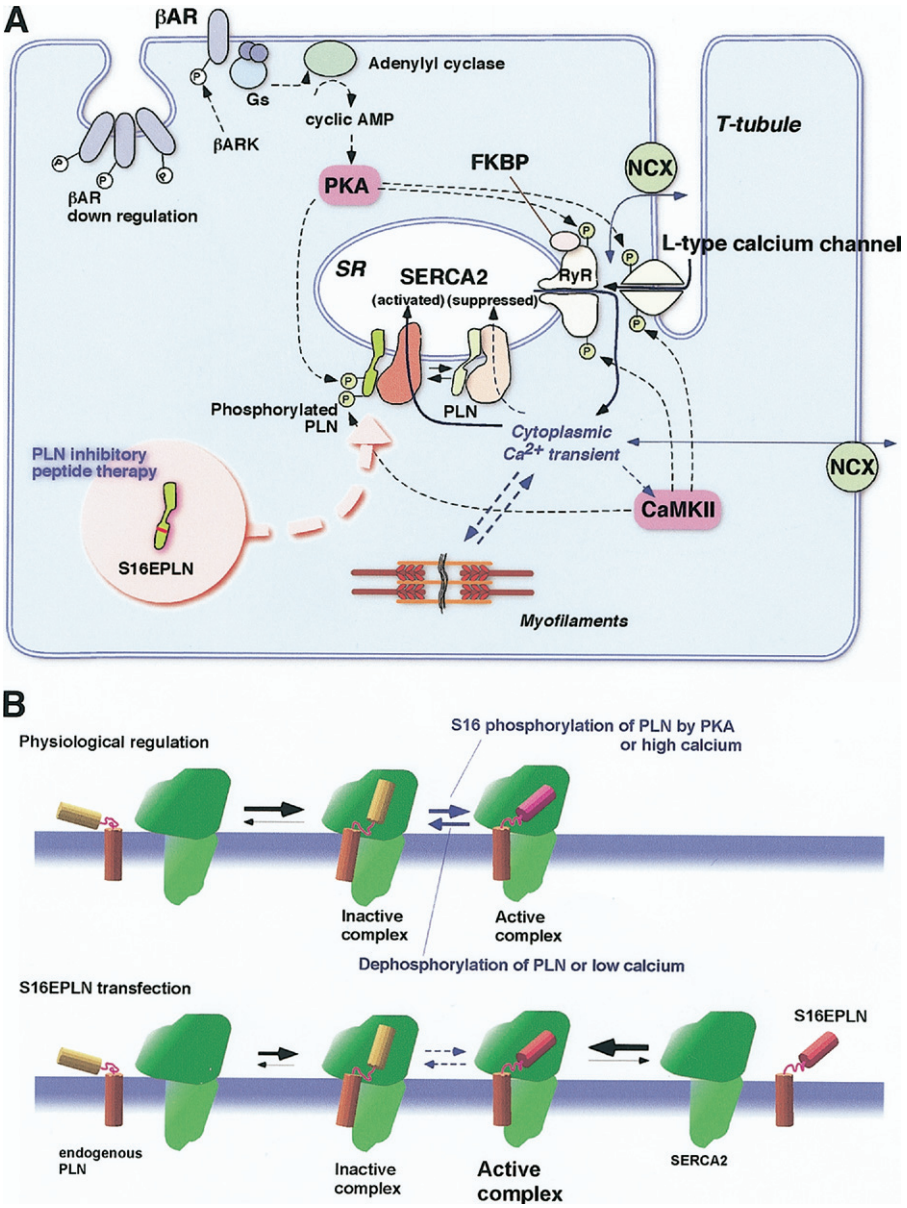

Figure 3. Regulatory systems of cardiac excitation-contraction coupling and the gene therapy targeting sarcoplasmic reticulum (SR) calcium uptake. **(A)** Intracellular mobilization of calcium ions governs myofilament contraction and relaxation. Two calcium release channels, the L-type calcium channel and ryanodine receptor (RyR), and a regulator of SR calcium uptake, phospholamban (PLN), are key controllers of calcium mobilization under the control of 2 second-messenger-regulated kinases, cyclic adenosine monophosphate (AMP)-dependent kinase (PKA) and calcium-calmodulin-dependent kinase II (CaMKII) (42). S16EPLN therapy directly targets SR calcium uptake (see text for details). **(B)** A working model of PLN-dependent SR calcium ATPase 2 (SERCA2) regulation has been refined, based on recent studies by Mueller et al. (61) and Zamoon et al. (62). Phospholamban is tightly bound to SERCA2 due to their low dissociation constant. Physiologically, high calcium concentration or PLN phosphorylation induces conformational changes of the cytoplasmic domain of PLN, which results in a structural rearrangement of PLN-SERCA2 interaction (from an inactive complex to an active complex) without dissociation. S16EPLN structurally resembles the Ser16-phosphoylated form PLN. Thus, S16EPLN transfection may stably constitute an active SERCA2-S16EPLN complex in cardiomyocytes. ARK = adrenergic receptor kinase; βAR = beta-adrenergic receptor; FKBP = K506 binding protein; NCX = Na-Ca exchanger.

thy phenotypes, while benefits were unclear in other model systems. Obviously there are limitations to the use of genetically based models to validate new therapeutic strategies for acquired forms of human heart failure. Indeed, most of these models have not been validated with respect to their fidelity of predicting therapeutic efficacy in the clinical setting. Furthermore, it would be pivotal to evaluate the effects of the reversal of the calcium cycling defect on heart failure after it had already been fully established as opposed to examining effects to prevent the initial onset. In this regard, as described in subsequent sections, we tested the PLN inhibitory therapy in 2 well-established and fully validated small animal heart failure models; one is a genetic form and the other is surgically induced, both of which have a long history of extensive use for a wide variety of pharmacological therapy. In both cases, PLN inhibition was

induced by cardiac-targeted somatic gene transfer (Fig. 3A), substantially after the onset of heart failure and during its disease progression, to allow a direct examination as to whether improvement of calcium cycling defects might lead to a reversal of components of the heart failure phenotype.

PLN INHIBITION RESCUES HEART FAILURE IN THE HAMSTER MODEL OF LIMB-GIRDLE MUSCULAR DYSTROPHY AND CARDIOMYOPATHY

To allow long-term, high efficiency, in-vivo transcoronary delivery and expression of foreign genes, we first developed a new transcoronary gene delivery system that utilized a recombinant adeno-associated virus (rAAV) vector (48). The therapeutic payload of the vector was a PLN inhibitory peptide, which consisted of a pseudo-phosphorylated mutant of PLN with a single amino-acid change at the position Ser16 to Glu (S16EPLN), designed to mimic the conformational change in PLN after phosphorylation by PKA (Fig. 3B). This S16EPLN/rAAV vector was then used to constitutively activate SR Ca^{2+} cycling in the myocardium of the well-characterized small animal model of chronic heart failure and DCM, the BIO14.6 cardiomyopathic (CM) hamster, which is based on a mutation in the δ-sarcoglycan gene. This genetic defect represents an autosomal recessive form of human myopathy found in limb-girdle muscular dystrophy type F (49). Although cardiac involvement of human limb-girdle muscular dystrophy is generally mild, δ-sarcoglycan deficiency in small animals including the BIO14.6 CM hamster and its derivatives and δ-sarcoglycan knockout mice causes an aggressive form of cardiomyopathy (50,51). Although there is an ongoing debate whether sarcolemmal defects in cardiomyocytes are a primary cause of δ-sarcoglycan-null cardiomyopathy or vascular abnormality contributes to its pathogenesis (52–54), sarcolemmal fragility may be a common pathological process of cardiomyopathies that are induced by genetic defects of cortical cytoskeletal proteins (52,53) or their secondary defects (55). Interstitial and replacement fibrosis also contributes to the pathological process of cardiomyopathy in BIO14.6 CM hamsters (52,53).

Nevertheless, the chronic inhibition of PLN by the S16EPLN peptide resulted in a marked enhancement of cardiac diastolic and systolic function even at advanced stages of the disease over 7 months from the initial delivery of the gene (48). Accordingly, the S16EPLN was capable of inducing the stable activation of SERCA2 and resulted in the enhancement of cardiac relaxation and subsequent contractility in the absence of adrenergic stimuli, which was analogous to the null phenotype of the PLN mutant mice (16).

PLN INHIBITION RESCUES HEART FAILURE DEVELOPMENT IN THE RAT MODEL OF POST-MYOCARDIAL INFARCTION (POST-MI) CHRONIC HEART FAILURE

The rAAV-mediated pseudo-phosphorylated PLN gene transfer therapy was tested in the chronic failing hearts of post-MI rats (56). Pre-gene transfer echocardiography documented that the recipient mouse (5 weeks after myocardial infarction surgery, with 30% to ~40% infarction size selected by echocardiography) had a moderate level of heart failure with the average percent fractional shortening as 32.4%, compared with the sham control animals (average percent fractional shortening, 53.1%). Eighty-five percent animals survived the gene transfer procedure, which was mostly similar to that used for the preceding BIO14.6 CM hamster studies, and the therapeutic prognosis was evaluated by repeated echocardiography over 6 months (Figs. 4A to 4F).

The left ventricular ejection fraction was enhanced by 6.0% at 2 months compared with the pre-gene transfer baseline value and by 12.8% at 6 months in S16EPLN-treated MI-rats, in contrast with the progressive decline of left ventricular ejection fraction in saline-treated control post-MI animals (Fig. 4C). Change in the left ventricular end-diastolic volume of S16EPLN-treated rat from the pre-gene transfer to the end point was limited to 18.2%, which was indistinguishable with sham rats and was considered proportional to the natural growth of animals (Fig. 4A), suggesting the complete suppression of progressive left ventricular dilation by the S16EPLN therapy in post-MI rats (note the left ventricles of saline-treated control post-MI animals continued to enlarge, as shown in Fig. 4A). Hemodynamic analyses further confirmed the improvement of left ventricular function by the S16EPLN/rAAV strategy (56). Among other improvements, the left ventricular end-diastolic pressure, which was considerably elevated in MI-saline rats, was near normal in the S16EPLN treatment group (56). In quantitative histologic analysis, a significantly lower extent of interstitial fibrosis (Fig. 4I) and suppression of cardiomyocyte hypertrophy were apparent in the non-infarct region in the S16EPLN-treated animals versus the control group (56). Taken together, these results indicate that enhancing calcium cycling by inhibiting PLN was capable of significantly enhancing multiple independent parameters of cardiac function even in the setting of the loss of over 30% of the viable myocardium.

CLINICAL APPLICATION OF PLN INHIBITION THERAPY AND OTHER THERAPIES TO ENHANCE SR CA^{2+} UPTAKE

Previous clinical studies questioned the relevancy of the use of inotropic agents to treat chronic heart failure. There was evidence that chronic exposure to positive inotropic agents, such as catecholamines or phosphodiesterase inhibitors, can lead to a long-term decrease in cardiac function and an associated decrease in survival (57). Furthermore, administration of beta-blockers is now a mainstream heart failure treatment together with the afterload reduction therapy with angiotensin-converting enzyme inhibitors, improving survival and the progression of clinical heart failure (58). As noted in the current review, we consider defects in calcium

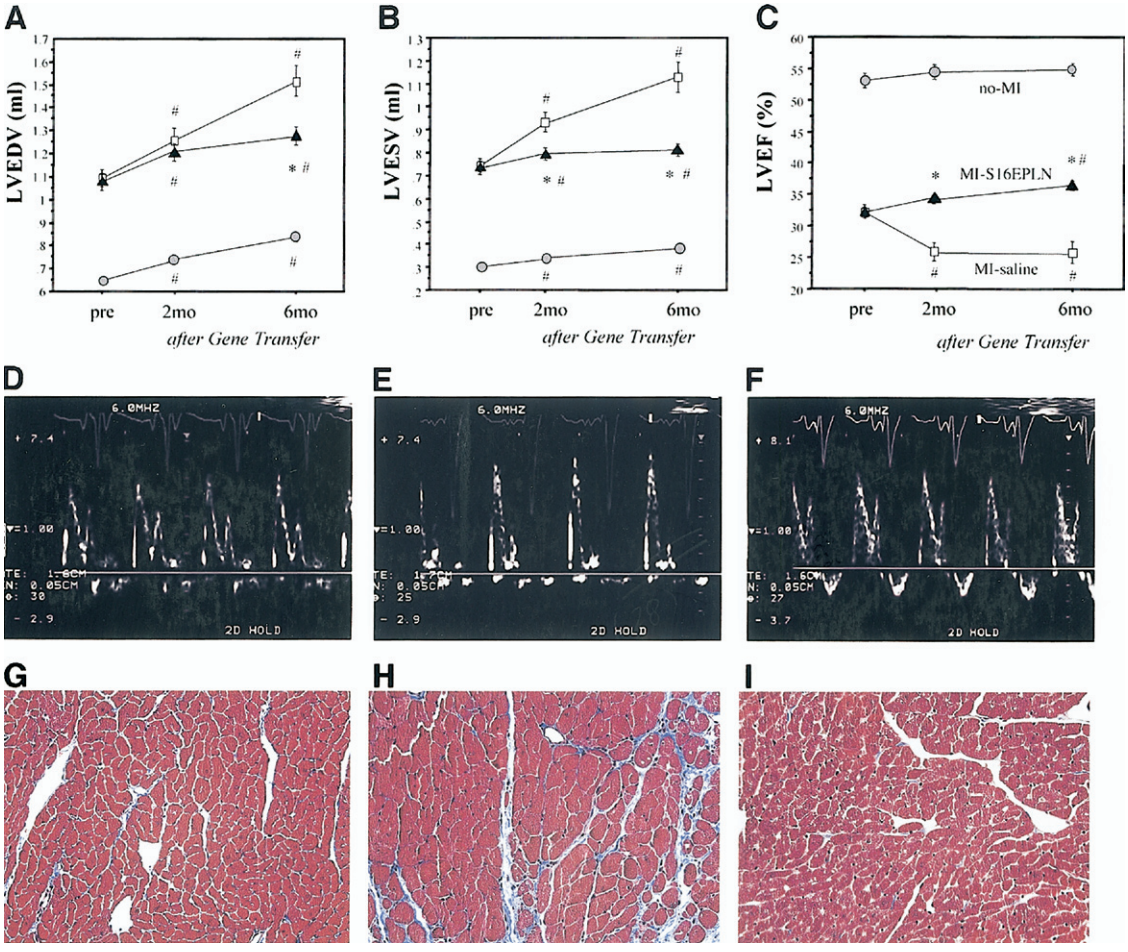

Figure 4. Chronic therapeutic effects of S16EPLN/rAAV-treatment in post-myocardial infarction (MI) rats. Serial changes of echocardiographic variables **(A to C)** before and after S16EPLN gene transfer and pulse-wave Doppler imaging of mitral flow found in sham (no-MI) rats **(D)**, saline-treated post-MI rats **(E)**, and S16EPLN-treated post-MI rats **(F)**. Note elevated early filling velocity and diminished late filling found in saline-treated post-MI rats were normalized in S16EPLN-treated animals. Suppression of the induction of interstitial fibrosis in the non-infarcted region in S16EPLN-treated animals was obvious **(G to I)**. **(G)** Sham (no-MI) rat myocardium; **(H)** Saline-treated post-MI rat myocardium; **(I)** S16EPLN-treated post-MI rat myocardium. Modified from figures in Iwanaga et al. (56). LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume.

cycling are a final common pathway for decreases in cardiac function in advanced forms of the disease, irrespective of the etiology. The unique aspect of S16EPLN/rAAV gene therapy, in contrast with the preceding pharmacologic inotropic agents, is that this therapy targets the most downstream point of the regulatory pathway of excitation-contraction coupling without chronic increases in cytoplasmic cyclic AMP levels or chronic PKA activation. Accordingly, chronic improvement of excitation-contraction coupling in a cyclic AMP-independent manner with a high efficiency, long-term, and cardiotropic S16EPLN/rAAV gene delivery appear to be a novel therapeutic strategy for slowing heart failure progression. The inherent cardiac and slow skeletal muscle specificity determined by the native distribution of PLN is another safety aspect of S16EPLN therapy for clinical applications. It should be also noted that a large body of independent work by del Monte and Hajjar (59) elegantly moved forward to work on the potential therapeutic role of SERCA2 gene therapy for advanced forms of heart failure, suggesting another approach to target defects in SR calcium cycling in heart failure.

To translate the SR calcium-targeted therapy to human clinical settings, we have been collaborating with Drs. David Kaye and John Powers of Baker Heart Institute in Melbourne, Australia, on the development of a novel clinically relevant, percutaneous gene delivery system with viral vectors (60). A unique strategy of safe, reproducible, high-efficient delivery of rAAV vectors (serotype 1 and serotype 2) to the myocardium of both healthy normal sheep and sheep with moderate-to-severe pacing-induced heart failure has been proven to be very reproducible. The catheter-based intracoronary SERCA2 or S16EPLN delivery involves recirculation of vectors in a closed-loop system, and this strategy has been shown to enhance cardiac contractility in heart failure in sheep.

These studies have set the stage for AAV gene therapy to enhance calcium cycling as a new therapeutic strategy for human heart failure, and the design of clinical studies is

moving forward in the near future. At the same time, the potential for developing small molecule inhibitors of PLN also needs to be reconsidered, given recent evidence that PLN actually represents an inhibitory subunit of SERCA (Fig. 3B), supported by recent elegant studies of Mueller et al. (61) and Zamoon et al. (62). Thus, the potential for the development of allosteric inhibitors of PLN remains a real possibility. New molecular-based strategies for enhancing calcium cycling appear to be on the horizon and may offer new hope for patients with advanced forms of the disease in the future.

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Reprint requests and correspondence: Dr. Kenneth R. Chien, Massachusetts General Hospital, Cardiovascular Research Center, Richard B Simches Research Center, CPZN 3208, 185 Cambridge Street, Boston, Massachusetts 02144-2790. E-mail: kchien@partners.org.

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